

a) Amendment to the Claims

Claims 1-3 (Cancelled).

4. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a microorganism having N-acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation

obtained by extracting the cells when a microorganism having N-acetylneuraminic acid synthetase activity is used in (i) above, (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA encoding N-acetylglucosamine 2-epimerase ~~derived~~ obtained from a microorganism belonging to the genus *Synechocystis*, or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the ~~aqueous~~ culture medium, and (iv) an energy source which is necessary for the formation of a pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

5. (Currently Amended) A process, for producing ~~N-acetylneuraminine~~ N-acetylneuraminic acid which comprises:

providing in aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid aldolase activity or N-acetylneuraminic acid synthetase activity, or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (ii) a culture of a microorganism capable of producing pyruvic acid

or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, a treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a microorganism having N-acetylneuraminic acid aldolase activity is used in (i) above, or a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells when a microorganism having N-acetylneuraminic acid synthetase activity is used in (i) above. (iii) N-acetylmannosamine which is produced by allowing a culture of a microorganism harboring DNA encoding N-acetylglucosamine 2-epimerase or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium, wherein said DNA encoding N-acetylglucosamine 2-epimerase is selected from the group consisting of: (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2, and (iv) an energy source which is necessary for the formation of pyruvic acid or phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

6. (Previously Amended) The process according to any one of claims 4, 5, 17 and 19, wherein said microorganism having N-acetylneuraminic acid aldolase activity is a microorganism belonging to the genus Escherichia or Corynebacterium.

7. (Previously Amended) The process according to any one of claims 4, 5, 18 and 20, wherein said microorganism having N-acetylneuraminic acid synthetase activity is a microorganism belonging to a genus selected from the group consisting of Escherichia, Neisseria and Streptococcus.

8. (Previously Amended) The process according to any one of claims 4, 5, 17 and 19, wherein said microorganism capable of producing pyruvic acid is a microorganism belonging to a genus selected from the group consisting of Escherichia, Corynebacterium and Saccharomyces.

9. (Previously Amended) The process according to any one of claims 4, 5, 18 and 20, wherein said microorganism capable of producing phosphoenolpyruvic acid is a microorganism belonging to a genus selected from the group consisting of Escherichia, Corynebacterium and Saccharomyces.

10. (Previously Amended) The process according to claim 6, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

11. (Previously Amended) The process according to claim 6, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

12. (Previously Amended) The process according to claim 7, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

13. (Previously Amended) The process according to claim 8, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

14. (Previously Amended) The process according to claim 9, wherein said microorganism belonging to the genus Escherichia is Escherichia coli.

15. (Previously Amended) The process according to claim 8, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

16. (Previously Amended) The process according to claim 9, wherein said microorganism belonging to the genus Corynebacterium is Corynebacterium ammoniagenes, Corynebacterium glutamicum or Corynebacterium acetoacidophilum.

17. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid adolase or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the

culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (iii) N-acetylmannosamine produced by allowing a culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity selected from the group consisting of (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2 or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-acetylmannosamine in the aqueous medium, and (iv) an energy source which is necessary for the formation of pyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

18. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid synthetase activity or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells. (ii) a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells. (iii) N-acetylmannosamine produced by allowing a culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity selected from the group consisting of (a) DNA encoding a protein having the amino acid sequence shown in SEQ ID NO: 1; and (b) DNA having the nucleotide sequence shown in SEQ ID NO: 2 or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells and N-acetylglucosamine to be present in an aqueous medium to form and accumulate N-

acetylmannosamine in the aqueous medium, and (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

19. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid adolase activity or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, an enzyme preparation obtained by extracting the cells, (ii) a culture of a microorganism capable of producing pyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (iii) a microorganism having N-acetylglucosamine 2-epimerase activity which carries DNA encoding N-acetylglucosamine 2-epimerase ~~derived~~ obtained from a microorganism belonging to the genus *Synechocystis*, and (iv) an energy source which is necessary for the formation of pyruvic acid;

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and



recovering N-acetylneuraminic acid from the aqueous medium.

20. (Currently Amended) A process for producing N-acetylneuraminic acid which comprises:

providing in an aqueous medium (i) a culture of a microorganism having N-acetylneuraminic acid synthetase activity or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (ii) a culture of a microorganism capable of producing phosphoenolpyruvic acid or a treated matter of the culture selected from the group consisting of concentrated culture, dried culture, cells obtained by centrifuging the culture, products obtained by treating the cells by various means such as drying, freeze-drying, treatment with a surfactant, ultrasonication, mechanical friction, treatment with a solvent, enzymatic treatment, protein fractionation and immobilization, and an enzyme preparation obtained by extracting the cells, (iii) a microorganism having N-acetylglucosamine 2-epimerase activity which carries a DNA encoding N-acetylglucosamine 2-epimerase ~~derived~~ obtained from a microorganism belonging to the genus *Synechocystis*, and (iv) an energy source which is necessary for the formation of phosphoenolpyruvic acid:

allowing N-acetylneuraminic acid to form and accumulate in the aqueous medium; and

recovering N-acetylneuraminic acid from the aqueous medium.

21. (Previously Amended) The process according to claim 17 or 19, wherein said culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity or treated matter thereof is copresent with said cultures of a microorganism having N-acetylneuraminic acid aldolase activity and said culture of microorganism capable of producing pyruvic acid or treated matter thereof.

22. (Previously Amended) The process according to claim 18 or 20, wherein said culture of a microorganism carrying DNA encoding N-acetylglucosamine 2-epimerase activity or treated matter thereof is copresent with said cultures of a microorganism having N-acetylneuraminic acid synthetase activity and said culture of microorganism capable of producing phosphoenolpyruvic acid or treated matter thereof.